



BRIEF REPORT

Body Adiposity of Young Male Rats Fed a Diet Containing Flaxseed Flour during Lactation or in Post-Weaning Period

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Abstract

Background: The aim of this study was to evaluate, at 90 days, the adipose tissue in young male rats fed with flaxseed flour during lactation or during post weaning period.

Methods: At birth, Wistar rats were assigned to two groups, whose dams fed with control (C) or flaxseed flour (F) diet during lactation. At 21 day, pups were weaned and fed control (CC and FC) or experimental diet containing flaxseed flour (CF). At 90 days, food and energy intake, body mass and length, body fat composition by dual-energy X-ray absorptiometry, leptin, insulin and intra-abdominal fat were analysed.

Results: CF showed ($P < 0.05$): Higher body mass, leptin, mesenteric and epididymal (vs. CC) fat mass. FC showed ($P < 0.05$): Lower total and trunk fat mass, intra-abdominal (vs. CF), retroperitoneal (vs. CC) fat mass and adipocyte area (vs. CF).

Conclusion: Flaxseed flour, during lactation period, contributes to lower intra-abdominal adiposity in young life.

Keywords

Fat tissue, Dual-energy X-ray absorptiometry, Experimental model

Introduction

The regulation of adipose tissue may be one critical pathway for controlling obesity, which can be carried via genetic predisposition, an individual's metabolism, physical activity, as well as by factors related to lifestyle, such as diet [1]. And the type of fat consumed could be more decisive than the total amount of fat in the diet to body composition and distribution of adipose tissue [2].

Regarding to essential fatty acids, alpha-linolenic acid (ALA) is associated with reduced obesity risk because exert numerous effects on adipose tissue [3]. In this context, flaxseed (*Linum usitatissimum*) can contribute to the prevention of obesity, since the seed is considered an excellent ALA source [4].

Previously, Costa, et al. [5] observed, in male rats, that diet containing flaxseed flour promotes adipose tissue down-regulation at 90 days. However, it was not explained if short and long intake of flaxseed flour displays similar effects on adipose tissue. So the study evaluate, at 90 days, the adipose tissue in young male rats fed with flaxseed flour during lactation or during post weaning period.

Methods

Protocol used for dealing with experimental animals was approved by the Federal Fluminense University Ethics Committee on Animal Research, Niteroi-RJ, Brazil (protocol 209/2012). All procedures were performed in accordance with the Brazilian Science and Laboratory Animal Society, and the Guide for the Care and Use of Laboratory Animals provisions published by the US National Institutes of Health (NIH Publication N 85-23, revised in 1996).

Wistar rats were kept in a temperature-controlled room (23 ± 1 °C), humidity ($60 \pm 10\%$), with artificial dark-light cycle (lights on from 7 a.m. to 7 p.m.). Virgin female rats were caged with male rats (3-months-old respectively). After mating, each female was placed in an individual cage with free access to water and standard laboratory food (Nuvilab®, Paraná, Brazil).

Within 24 hours of birth, excess pups were removed, and only six male pups were kept per dam, which maximizes lactation performance [6]. During lactation period, pups were randomly assigned: Control (C, $n = 24$), whose dams were fed with control diet containing 20 g casein, 52.95 g cornstarch, 7 g soybean oil and 5 g fiber per 100 g; experimental (F, $n = 12$), whose mothers were fed with diet containing 25 g flaxseed flour, 45.84 g cornstarch and 15 g casein per 100 g. Diets have same amounts of sucrose (20 g), mineral (3.5 g) and vitamin mix (1 g), L-cystine (0.3 g) and choline bitartrate (0.25g), per 100g [7]. During 21 days of lactation, free access to water and diets was not evaluated, due to difficulties in controlling pup food intake. Diets were manufactured and stored in pellets at 4 °C in agreement with American Institute of Nutrition (AIN-93G) recommendations for rodent diets [8]. Flaxseed flour 25g per 100g aimed to meet the entire recommended fiber intake; adding oil was not necessary, as this seed comprises a source of such component [4].

At 21 days, pups were weaning and fed a control diet (CC group, $n = 12$ and FC group, $n = 12$) or experimental diet containing 25g per 100g flaxseed flour (CF group, $n = 12$), respectively. The animals were housed in collective plastic cages. Food (g) and energy intake (k cal) were evaluated weekly. At the end of the nutritional period, at 90 days, body mass (g) and length (cm, measured as distance between nose tip to tail tip) were evaluated.

After 8 hours of fasting, rats were anesthetized with Thiopentax (Sodium thiopental, 0.1 mg per 100g) and subjected to dual-energy X-ray absorptiometry (DXA), using a Lunar DXA 200368 GE instrument (Lunar, with specific software encore 2008 version 12.20, GE Healthcare, Madison, Wisconsin, USA). Total fat mass (g) and trunk fat mass (g) were measured for each rat.

Blood was collected by cardiac puncture following DXA procedures. Samples were centrifuged, and serum stored at -80 °C for later analysis. Leptin and insulin (pg

ml⁻¹, respectively) were measured using multiplex assay kits (Millipore rat panel RBN1MAG-31K-03, Billerica, MA, USA). Glucose (mg dL⁻¹) was measured by colorimetric method (Bioclin BS-120, Belo Horizonte, MG, Brazil).

Intra-abdominal adipose tissue (retroperitoneal, mesenteric and epididymal) was dissected and weighed (g). Retroperitoneal fat was collected and fixed in buffered formaldehyde. Tissues were embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin-eosin (HE). Profiles with at least 100 adipocytes were randomly selected and captured for morphometric analyses. Sectional adipocytes area (μm²) was determined on digital images acquired with an Optronics CCD video camera system and Olympus BX40 light microscope, analysed with U.S. National Institutes of Health IMAGE-J software <http://rsbweb.nih.gov/ij/>.

Statistical analyses were performed using Graph-Pad Prism statistical package version 5.0, 2007 (San Diego, CA, US). Food and energy intake were analysed using two-way variance analysis (ANOVA), followed by Bonferroni post-test. Remaining results were analysed using one-way analysis of variance, followed by Newman-Keuls post-test. All results are expressed as means ± SEM (standard error of the mean) with a 0.05 significance level.

Results

Food, energy intake and body length were similar between groups. However, the body mass was higher ($p < 0.05$, CC: 400.90 ± 11.58 ; CF: 431.50 ± 8.50 and FC: 383.90 ± 10.45 g) in CF group (vs. CC and FC) (Figure 1).

Body adiposity analysis, by DXA, showed lower ($p < 0.05$) total and trunk fat mass in FC group (vs. CC and CF). Intra-abdominal (vs. CF) and retroperitoneal (vs. CC) fat mass were lower ($p < 0.05$) in FC group. Mesenteric (vs. CC and FC) and epididymal (vs. CC) fat mass were higher ($p < 0.05$) in CF group. Regarding serum analysis, insulin and glucose were similar between groups, however, leptin concentrations were higher ($p < 0.05$) in CF group (vs. CC and FC). Retroperitoneal adipocytes area were lower ($p < 0.05$, vs. CF) in FC group (Table 1).

Discussions

In present study, ALA provided by flaxseed (*Linum usitatissimum*) flour during lactation or during post-weaning period, showed differences in body and intra-abdominal fat mass at 90 days. Lucas [9] suggested that programming stimulus exerts long-term effects when applied at sensitive period. In fact, flaxseed flour in early period of life was more relevant for prevention of higher body adiposity because after weaning, FC group was fed with diet containing linoleic acid (LNA) provided by soybean oil. LNA can induce obesity, mainly increasing rate adipocytes differentiation and hypertrophy [10]; however, the FC group displayed lower fat

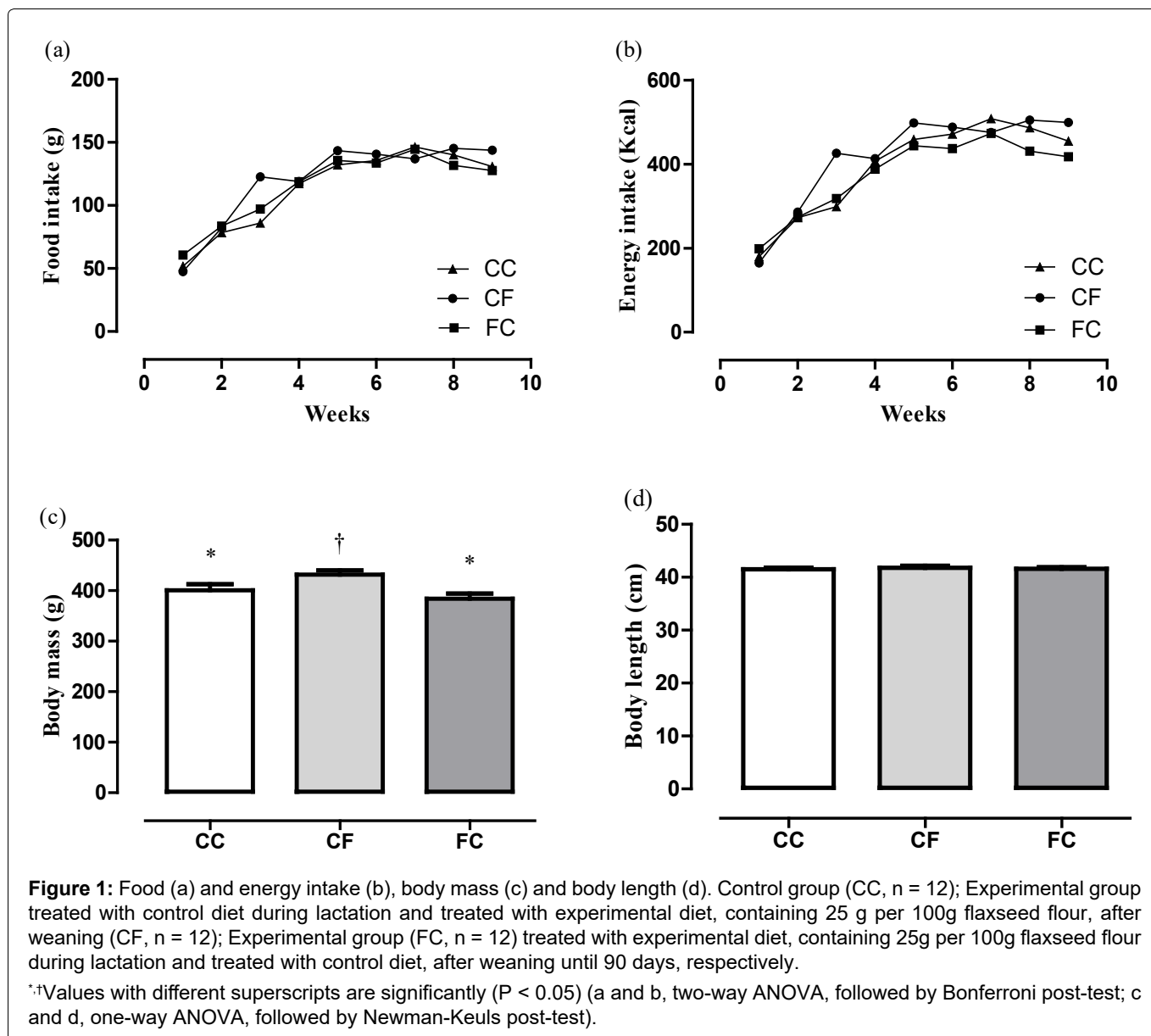


Table 1: Body adiposity composition, intra-abdominal fat mass and serum analyses at 90 days.

	CC		CF		FC	
	Mean	SEM	Mean	SEM	Mean	SEM
Total fat mass (g)	145.30 [*]	10.08	151.80 [*]	8.71	109.80 [†]	7.27
Trunk fat mass (g)	99.55 [*]	7.30	95.83 [*]	5.18	75.08 [†]	6.58
Intra-abdominal fat mass (g)	28.10 [*]	3.13	29.92 [†]	2.12	21.66 ^{†*}	1.58
Retroperitoneal fat mass (g)	12.65 [*]	1.50	11.46 [†]	0.97	8.46 [†]	0.84
Mesenteric fat mass (g)	5.84 [*]	0.55	7.52 [†]	0.54	4.76 [*]	0.41
Epididymal fat mass (g)	7.26 [*]	0.80	10.93 [†]	1.43	8.42 [†]	0.60
Leptin (pg ml ⁻¹)	5392 [*]	901.50	8220 [†]	731.20	4439 [*]	842.80
Insulin (pg ml ⁻¹)	683.10	184.70	771.00	188.20	405.90	80.18
Glucose (mg dl ⁻¹)	119.30	7.11	117.10	6.20	110.30	7.66
Adipocytes area (μm ²)	4477 [*]	331.8	5279 [†]	414.5	3448 ^{†*}	337.9

Control group (CC, n = 12); Experimental group treated with control diet during lactation and treated with experimental diet, containing 25 g per 100 g flaxseed flour, after weaning (CF, n = 12); Experimental group (FC, n = 12) treated with experimental diet, containing 25 g per 100g flaxseed flour during lactation and treated with control diet, after weaning until 90 days, respectively. Results are expressed as means ± SEM.

*,†Significantly different from the control group (one-way ANOVA, followed by Newman-Keuls post-test, $P < 0.05$). SEM, standard error of the mean.

mass and lower adipocyte area. Serum leptin concentrations help to consolidate these findings because lower circulating leptin is correlated with lower body fat in human and rodents [11].

Adiposity can also be associated with insulin concentration [12] and previous report that ALA prevents high insulin release [13]. Martin, et al. [14] observed that ALA during pregnancy and lactation improving glucose tolerance, insulin sensitivity and reducing the fat mass stores in induced obese mice. However, Heskey, et al. [15] related that higher adipose tissue ALA was inversely associated with insulin resistance. Although in present study the groups displayed no statistical difference to insulin, we highlight the higher serum concentrations of this hormone in the CF group (+12% vs. CC and +90% vs. FC). Further studies are necessary to evaluate if ALA long intake, after weaning, is associated with higher insulin and intra-abdominal adiposity, as observed in the CF group.

Considering that early period of life is fragile target to environmental aggression, which can induce to high body adiposity in later in life [15], our experimental data suggests that flaxseed (*Linum usitatissimum*) flour, during lactation period, contributes to lower intra-abdominal adiposity in young life.

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Author Contribution

MDC Abreu, LR Pessoa, BFC Boueri and CR Pessanha treated the animals during the experimental period and performed the dual-energy X-ray absorptiometry; AD Pereira and DC Ribeiro performed serum and intra-abdominal fat mass analyses; AS Santos and CCA Nascimento-Saba performed morphometric analyses; CAS Costa and GT Boaventura analyzed results and wrote the final version of manuscript.

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